Chapter 19
Induction of Plant Disease Resistance and Other Physiological Responses by Green Light Illumination

Rika Kudo and Keiji Yamamoto

Abstract Research on lighting as part of integrated pest management (IPM) has been attracting attention recently. This chapter presents the authors' research regarding the effects of green light on defense reaction in plants against plant pathogens, such as the induction of disease resistance in plants, endogenous production of antibacterial substances, and reinforcement of cell walls through elevation of the levels of lignin which is a main component of cell walls. This chapter also shows how green light, which had generally been regarded as having little use in plants, is effective not only in disease control but also in spider mite suppression, promotion of plant growth, and increase in functional substances. It describes how the authors developed green LED light sources for disease resistance induction and conducted experiments on major horticultural crops such as strawberries, tomatoes, perillas, and garlic chives, considering their economic benefits and practicality.

Keywords Green light • Disease resistance induction • Defense reaction • Elicitor • Strawberry anthracnose (*Glomerella cingulata*) • Spider mite control • Quality improvement • Dormancy suppression • Green LED

19.1 Introduction

As a growing number of people seek food safety and security, the need for cultivation methods that use no or reduced amounts of agricultural chemicals is rising. On the other hand, disease and pest damage should be controlled for stable production at cultivation sites. This has given rise to a significant need for new disease control techniques that can replace agricultural chemicals.

To satisfy those requirements, the use of integrated pest management (IPM) is spreading, combining agronomic, biological, and physical controls while reducing

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environmental impact by limiting spraying of agricultural chemicals. Yellow or green moth repellent lights are examples of IPM used to suppress the activities of owlet moths.

Plants activate various defense reactions when exposed to stresses, such as disease damage, insect damage, and temperature- and water-related stresses. Substances that induce disease resistance reaction in plants are called elicitors, which can be divided into biological elicitors such as molecules in the cell wall of pathogenic microbes and nonbiological elicitors such as heavy metals and surface-active agents (Robatzek and Somssich 2001). It has been reported that red light and ultraviolet light act as nonbiological elicitors (Islam et al. 1998; Arase et al. 2000; Kobayashi et al. 2013).

This chapter focuses on green light as a nonbiological elicitor while considering its safety in greenhouses and describes the authors' findings on the induction of disease resistance and other physiological responses of plants by green light illumination, which has mostly escaped attention until now in studies on the use of visible light on plants.

19.2 Induction of Disease Resistance by Green Light Illumination

19.2.1 Effects of Light Quality on Gene Expression Related to Disease Resistance

Light-emitting diodes (LEDs) were used to illuminate (or irradiate) various wavelengths of the visible light spectrum on tomato seedlings, and the expression of various genes related to disease resistance was examined (Table 19.1). As a result, the expression of allene oxide synthase (AOS) genes, which are necessary for biosynthesis of jasmonic acid, a type of plant hormone that is considered to be related to disease resistance, was specifically observed only when the seedlings were illuminated with green light (Kudo et al. 2009) (Fig. 19.1).

Increased expression of lipoxygenase (LOX) genes, which, similarly to the AOS genes, are related to biosynthesis of jasmonic acid, and induction of gene expression of chitinase and various other pathogenesis-related (PR) proteins were also observed with illumination of green light (Kudo et al. 2009) (Figs. 19.2 and 19.3).

Furthermore, genes that increased their expression as a result of green light illumination on rice and strawberries were examined using the deoxyribonucleic acid (DNA) microarray. The results showed that green light illumination increased expression of heat shock proteins and other stress-related genes as well as osmotin-like proteins and various genes related to disease resistance.

These results showed that green light illumination, which had mostly been disregarded due to the perception that it contributes little to plant photosynthesis, has an effect on the expression of genes related to plant disease resistance. It is

Table 19.1 Wavelength range and peak wavelengths of LED light sources

LED light	Blue	Green	Yellow	Red
Wavelength range (nm)	400–500	480–590	560–650	560–790
Peak wavelength (nm)	470	520	590	660

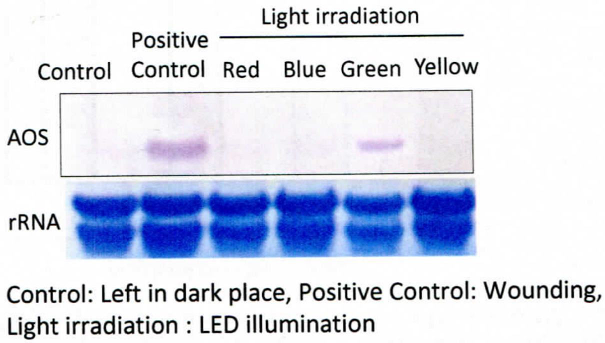

Fig. 19.1 Effect of light quality on induction of tomato AOS gene expression by light illumination (Kudo et al. 2009). Cultivar, "Momotaro 8" (Takii & Co., Ltd.); air temperature, 25 °C for 2 weeks to raise the seedlings; LED light sources, blue, green, yellow, and red. Illumination: 2 h from 1 cm above the top of the plant body of the tomato seedlings. The tomato leaves were collected immediately after the illumination and chilled using liquid nitrogen and ground to powder. RNA was extracted from the powder, and analysis was carried out on allene oxide synthase (AOS) genes by northern blotting

assumed that green light illumination causes moderate stress in plants, which prompts induction of disease resistance (Kudo et al. 2009).

Based on the above findings, a system was created in which diseases are caused artificially at the probability of 100 % to assess, at a laboratory level, the effects of green light illumination in controlling diseases in major horticultural crops. The results showed that green light illumination was effective in controlling some of the main diseases in horticultural crops, namely, strawberry anthracnose caused by *Glomerella cingulata*; gray mold caused by *Botrytis cinerea* in tomatoes (*Lycopersicon esculentum* Mill.), peppers (*Capsicum annuum* L.), and eggplants (*Solanum melongena* L.); damping-off of spinach caused by *Pythium ultimum*; and corynespora leaf spot disease in perillas caused by *Corynespora cassiicola* (Kudo et al. 2010).

As the next step, an experiment was conducted, with the cooperation of greenhouse growers, on the crops for which green light illumination was observed to be effective at the laboratory level to verify its effect in controlling diseases in horticulture. Incidentally, the authors are not sure which term is more appropriate, illumination (effects of photons with particular wave numbers) or irradiation (effects of energy with particular wavelength).

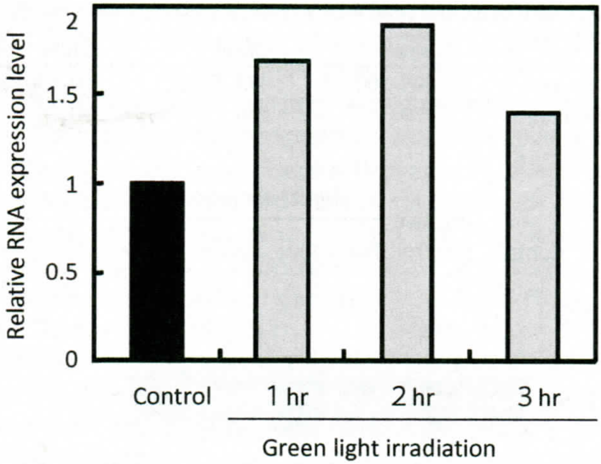

Fig. 19.2 Effect of green light illumination period on induction of tomato LOX gene expression (Kudo et al. 2009). Cultivar: "Momotaro 8" (Takii & Co., Ltd.). The tomato seedlings were raised in a nursery room at a temperature of 25 °C under fluorescent lamps for 2 weeks. Green light illumination: 80 μmol m^{-2} s^{-1} for 1, 2 or 3 h at 1 cm above the top of the tomato seedlings. The tomato leaves were collected immediately after the illumination and chilled using liquid nitrogen and ground to powder. Ribonucleic acid (RNA) was extracted from the powder and analysis was carried out on lipoxygenase (LOX) genes by real-time polymerase chain reaction (PCR)

19.2.2 Effects of Green Light on Strawberry Anthracnose

There is a substantial need for controlling strawberry anthracnose at cultivation sites, and so the effects of green light illumination on strawberry anthracnose were examined. Strawberry anthracnose occurs particularly during a period of high temperature and high relative humidity while raising strawberry seedlings or after the seedlings are planted in the field, causing blighting of strawberry roots. It is highly infectious and develops rapidly. New types of strawberry anthracnose resistant to agricultural chemicals have emerged, and the risk of strawberry anthracnose is rising with the recent global warming.

After irradiating various colors of LED on strawberry tissue culture seedlings for 2 h, the leaf surface of the seedling was inoculated with a conidial suspension of strawberry anthracnose pathogen (*Glomerella cingulata*) and cultivated in a growth chamber under given conditions. After 2 weeks, the leaf surface was examined for strawberry anthracnose lesions. The results showed that whereas illumination of blue, red, or yellow light was not effective in controlling the development of lesions, only green light illumination was effective in significantly controlling the development of lesions (Fig. 19.4). The optimal conditions of green light illumination for disease control have so far been found to be photon flux density of 80 μmol m^{-2} s^{-1}, illumination time of 2 h at night, and illumination interval of once every 3 days (Kudo et al. 2011).

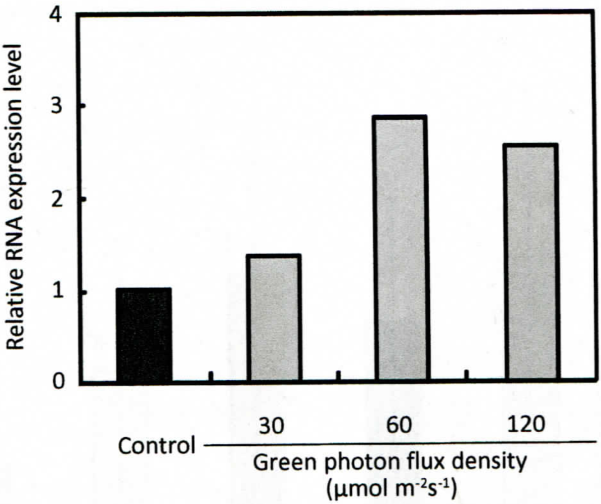

Fig. 19.3 Effect of green light illumination on induction of cucumber chitinase gene expression (Kudo et al. 2009). Cultivar: "Alpha Fushinari" (Kurume Vegetable Breeding Co., Ltd.). The seedlings were raised in a nursery room at a temperature of 25 °C under fluorescent lamps for 2 weeks. Green light illumination for 2 h: 30, 60 and 120 μmol m^{-2} s^{-1} at 1 cm above the top of cucumber seedlings. The cucumber leaves were collected immediately after the illumination and chilled using liquid nitrogen and ground to powder. RNA was extracted from the powder and analysis was carried out on chitinase genes by real-time PCR

Next, to ascertain the practicality of this technique, green light was irradiated to strawberries using LED lamps, fluorescent lamps, and metal halide lamps at the above-described illumination conditions during the raising of strawberry seedlings and cultivation, and a comparison was made with strawberries in plots without illumination in the incidence of strawberry anthracnose. The experiments were conducted in several locations in each of the four prefectures of Shikoku island, Japan. The results showed that the incidence of strawberry anthracnose declined at each test site by a third to two thirds (Kudo et al. 2011). Also, a simple diagnosis by ethanol immersion (Ishikawa 2013), a method for diagnosing latent infection by strawberry anthracnose, was used to examine changes in the incidence of latent infection by strawberry anthracnose. The results showed that green light illumination tended to control latent infection and that it was effective in reducing the incidence risk.

These findings suggest that the use of green light illumination during management and growth of the parent plants and during the raising of the seedlings may reduce the subsequent incidence of the disease.

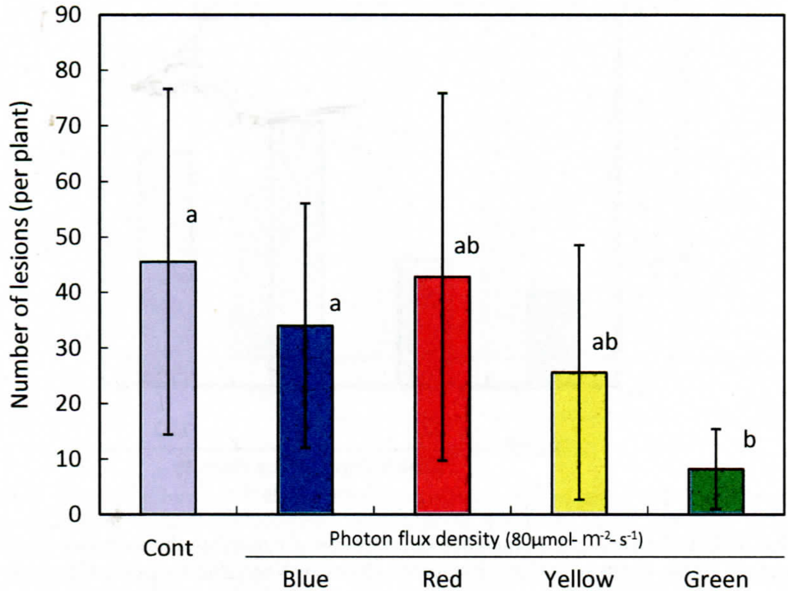

Fig. 19.4 Effect of light quality on the incidence of strawberry anthracnose (Kudo et al. 2010). Cultivar: "Smile Heart" (Shikoku Research Institute Inc.). LED light sources: blue, green, yellow, and red. Photon flux density: 80 μmol m^{-2} s^{-1}. The tissue-cultured transplants were illuminated for 2 h in an incubator set at 25 °C. A suspension of strawberry anthracnose pathogen was inoculated on the seedlings immediately by dispersion after illumination. The seedlings were then cultivated at 25 °C in an incubator for 2 weeks, after which the seedlings were assessed for incidence of strawberry anthracnose. *Based on Tukey's multiple comparison procedure, there are significant differences at the 5 % level between the English letters ($n = 5$)

19.2.3 Effects of Green Light on Corynespora Leaf Spot Disease

Perilla (*Perilla frutescens* L.), also known as Japanese herb in Japan, is a food ingredient in demand throughout the year for use in sashimi (raw fish). When symptoms of corynespora leaf spot disease (*Corynespora cassiicola*) appear in perilla leaves, the leaves lose entire commercial value and are discarded, causing a substantial decrease in yield. The paucity of registered agricultural chemicals for controlling disease in perilla makes it very difficult to control corynespora leaf spot disease in perilla.

Against this backdrop, perilla was cultivated using green LED light instead of the customary incandescent lamp as a source of artificial light for controlling plant growth in greenhouses. The results showed that green light illumination had a similar effect as incandescent lamps in suppressing bolting and that it was also effective in suppressing corynespora leaf spot disease (Kudo and Yamamoto 2013) (Fig. 19.5).

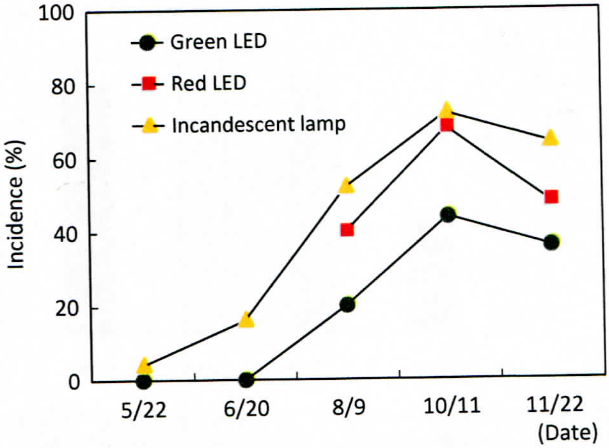

Fig. 19.5 Effect of light quality on the incidence of corynespora leaf spot disease (Kudo and Yamamoto 2013). Date of starting illumination, April 24, 2012; ending date of experiment, November 27, 2012. Perilla seedlings were planted in the plastic greenhouse for soil cultivation on March 20, 2012, and were subjected to the test using artificial lighting to control plant growth. Starting on April 24, perillas were illuminated from 22:00 to 23:30 every day using incandescent lamps, red LEDs, and green LEDs. The LEDs were placed 2 m apart. The photon flux density at the growth point under 1 m from LEDs was 0.5–1.0 μmol m^{-2} s^{-1}

Illuminating perilla with green LED light reduced the loss of perilla leaves due to disease and increased the yield and also reduced the frequency of agrichemicals application by half.

19.3 Various Effects of Green Light

While examining the effects of green light on controlling diseases in strawberry, perilla, and other crops in greenhouses, it became evident that green light had other effects on plants as well. Although green light had been regarded as having little effect on plants, it became clear that with the right illumination conditions, green light influenced plants' morphogenesis and had benefits on greenhouse horticulture. Specifically, in demonstration tests conducted in commercial greenhouses, it was discovered that green light not only was effective in controlling plant diseases but was also effective in controlling spider mites, promoting growth (fruit enlargement), improving quality, and controlling plant growth as an artificial light source.

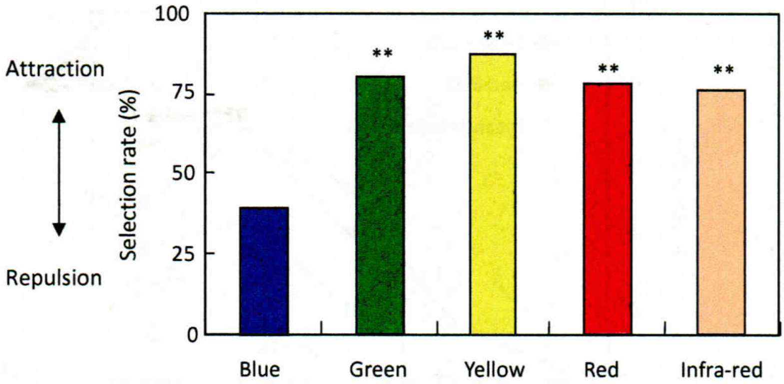

Fig. 19.6 Effect of light quality on the behavior of the predatory mite *Amblyseius* (*Neoseiulus*) *californicus* ($n = 30$). No significant tendencies were observed in the experiment on the effect of light illumination on phototaxis of spider mites. Therefore, another study was conducted on the effect of light illumination on phototaxis of the predatory mite (*Amblyseius* (*Neoseiulus*) *californicus*), which is used as a natural enemy of spider mites, using LEDs (blue, green, yellow, and red), at the photon flux density of 40 μmol m^{-2} s^{-1}. ** Significantly different at the 1 % level

19.3.1 Spider Mite Control

The phototaxis of phytoseiid mite (*Phytoseiidae*), which is a natural enemy of the spider mite, was examined. The results showed that phytoseiid mites move toward green light and light with longer wavelengths (Fig. 19.6). This finding suggests that when phytoseiid mites are released as a natural-enemy pesticide against spider mites, green light promotes settlement of the phytoseiid mites into the plants irradiated with green light and more intensive preying on spider mites.

19.3.2 Growth Promotion

It was found that illumination of green light during strawberry cultivation induced growth-related gene expression of cellulose synthase 2 and extensin (Fig. 19.7). Green light illumination was also observed to enlarge the leaf area during cultivation. TTC analysis at the end of the cultivation also revealed increased root activity from green light illumination (Figs. 19.8 and 19.9).

While it was believed that green light was not easily absorbed by plants, research in recent years has reported absorption of green light in plants and the use of green light for CO_2 fixation in mesophyll and for photosynthesis (Terashima et al. 2009; Sun et al. 2009). In addition to promotion of photosynthesis where green light acts as a signal for growth-related genes, there is a potential for use of green light to more directly promote photosynthesis. It is assumed that increased root activity also

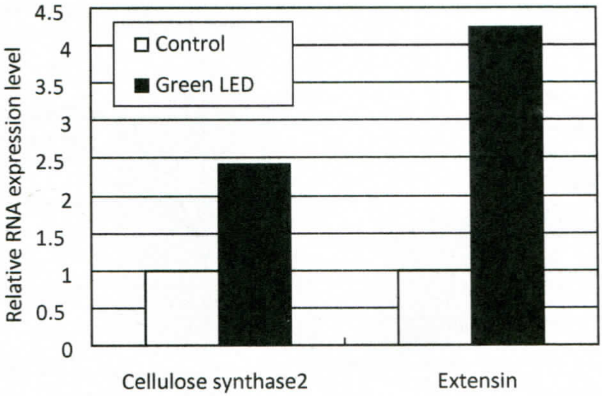

Fig. 19.7 Effect of green light illumination on expression of genes related to strawberry growth. Date of starting illumination, October 24, 2012; ending date of test, May 20, 2012. Illumination: 2 h at night, three times a week. The RNA samples extracted from the third leaf from the top of the strawberry *Sachinoka*, which was cultivated in the plastic greenhouse with illumination of green LEDs, were used to analyze induction of gene expression by real-time PCR. The samples were collected in February

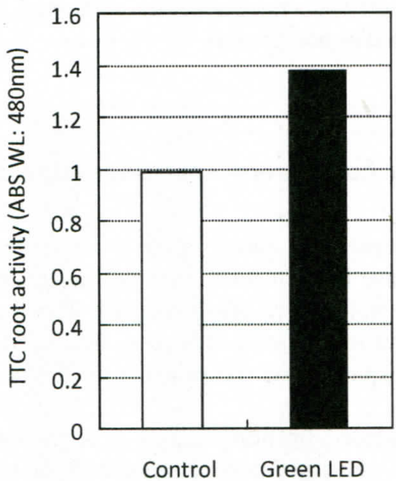

Fig. 19.8 Improvement in TTC root activity of strawberries by green light illumination. Date of starting illumination, October 24, 2012; ending date of test, May 20, 2012. Illumination: 2 h at night, three times a week. The root samples of the strawberry *Sachinoka*, which was cultivated in the plastic greenhouse with illumination of green LEDs, were used at the end of the cultivation to analyze root activity by triphenyl tetrazolium chloride (TTC) staining. The samples were collected in May

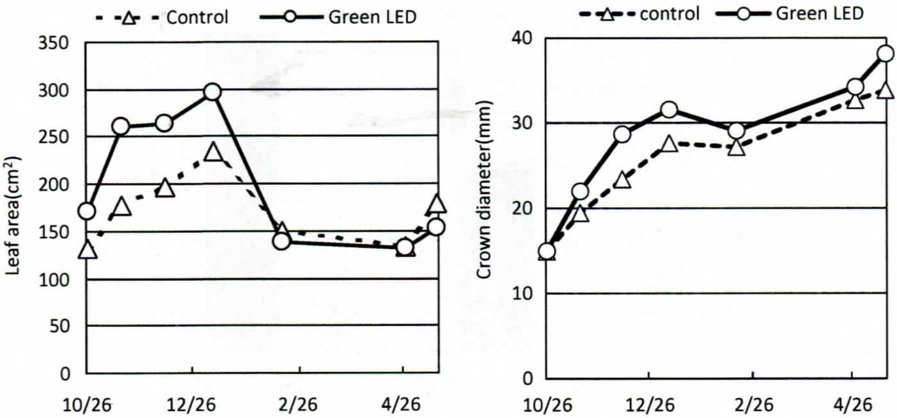

Fig. 19.9 Effect of green LED light illumination on strawberry leaf area and crown diameter. Date of starting illumination, October 24, 2012; ending date of test, May 20, 2012. Illumination: 2 h at night, three times a week. The data show changes in the leaf area of the third leaf from the top and the crown diameter of the strawberry "Sachinoka" during cultivation. "Sachinoka" was cultivated in the plastic greenhouse with illumination of green LEDs

resulted in increased nutrient absorption, the combined effect of which led to promotion of photosynthesis and growth.

19.3.3 Increase in Functional Substances and Sugar Content

Improvement in the sugar-acid ratio, which is an indicator of the quality of strawberries, and increase in such functional substances as total polyphenol and vitamin C in leaf vegetables were also observed. The total polyphenol content increased in perillas and leaf lettuces that were irradiated with green light. The increase in vitamin C in spinach and lycopene in tomatoes was observed after green light illumination.

Polyphenols are produced by biosynthesis as a secondary metabolite of the shikimic acid pathway. Phenylalanine ammonia-lyase (PAL) is an important enzyme that catalyzes the branching reaction from the primary metabolism to the secondary metabolism in the shikimic acid pathway of plants. It became clear that green light illumination stimulated PAL activity in leaf lettuces and increased the total polyphenol content (Figs. 19.10 and 19.11)

As for strawberries, green light illumination increased sugar content in winter. It is assumed that the rise in sugar content during the low-temperature season was due to the activation of a defense reaction to increase resistance against stress. The green light may have acted as a stressful stimulus to help plants accumulate functional substances (polyphenols, vitamin C, lycopene) and sugar as part of their resistance reaction.

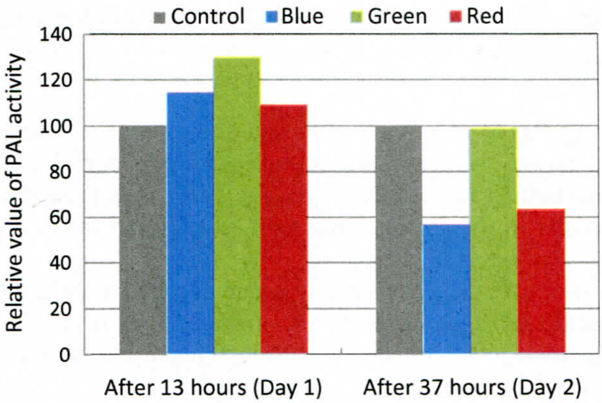

Fig. 19.10 Effect of light quality on PAL activity of leaf lettuces. After the seedlings of the leaf lettuce "Banchu Red Fire" were raised for 20 days, the seedlings were planted on a hydroponic styrofoam panel and cultivated in a nutrient flow technique (NFT) system in a plastic greenhouse. Fluorescent lamps (blue, green, and red) were used as the light source to illuminate at the photon flux density of 65 μmol m^{-2} s^{-1} for 2 h from 20:00 to 22:00 at the interval of once every 3 days. The PAL activity was measured 28 days after planting based on the Koukol-Conn method ($n=6$)

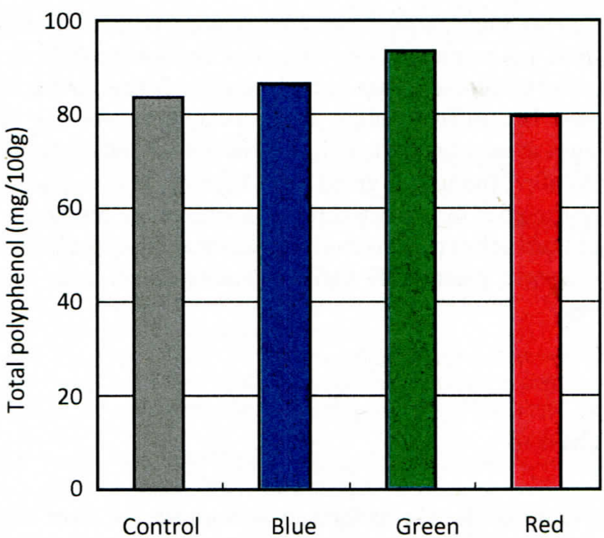

Fig. 19.11 Effect of light quality on polyphenol content of leaf lettuces. After the seedlings of the leaf lettuce "Banchu Red Fire" were raised for 20 days, the seedlings were planted on a hydroponic styrofoam panel and cultivated in a NFT system in the plastic greenhouse. Fluorescent lamps (blue, green, and red) were used as the light source to irradiate light at the photon flux density of 65 μmol m^{-2} s^{-1} for 2 h from 20:00 to 22:00 at the interval of once every 3 days. The total polyphenol content was measured ($n-3$) 45 days after planting based on the Folin-Denis method

19.3.4 Dormancy Suppression, Flower Bud Differentiation, and Bolting

Green LED light was used in place of incandescent lamps and fluorescent lamps, which are the conventional artificial light sources used for dormancy suppression in strawberries and bolting suppression in perillas. The results showed that green light had similar effects as the conventional light sources and induced photoperiodic responses in plants (Sun et al. 2009).

Perilla is a short-day plant, forming a raceme in summer and autumn on a stem that has differentiated from the leaf axil. Artificial lighting is used in yearlong cultivation of perillas to suppress bolting through photoperiodic adjustment and to enable continuous harvesting of the perilla leaves.

Green LED light was used instead of the customary incandescent lamp to study its effect on suppressing bolting of perillas. The results showed that green light had a similar effect in suppressing bolting as the incandescent lamp (Kudo and Yamamoto 2011, 2013).

In the winter, the growth of garlic chives is suppressed due to dormancy under the conditions of low temperature and short day. It was found that the illumination of green LED light on garlic chives that had not been subject to the use of artificial light to control their growth substantially promoted growth and increased yield.

On the other hand, when garlic chives are irradiated with incandescent lamps and other light sources that contain a large amount of red wavelengths to create a long-day condition, flower buds develop in early spring. It was found that green light suppressed flower bud differentiation and development. As this is perceived as having advantages in increasing yield during the winter and controlling diseases in early spring and after, the use of green LED lighting has been widely introduced recently in major regions in Japan where garlic chives are produced.

As green light can adjust photoperiodic flower bud differentiation and dormancy and suppress diseases, green LED light is gaining attention as a new source of artificial lighting.

19.4 Conclusion

In an effort to promote the use of light in agriculture, we have been developing "pest management by lighting" techniques in integrated pest management (IPM). As a result, it was found that the illumination of green light on plants enhanced plants' intrinsic disease resistance and prevented infection and incidence of diseases. It also became clear that green light illumination on horticultural products was not only effective in disease control but was also effective in spider mite control, promotion of growth (fruit enlargement), and quality improvement. Green light illumination does not require registration such as agricultural chemicals do, and there are no issues related to safety. For these reasons, green light illumination is considered to have substantial value as a new type of technique using light.

At present, we are working on commercializing green LED lighting sources (product name: Midorikikuzo®) and on establishing horticultural production technology with reduced environmental impact by combining the use of natural enemies, which are key components of IPM, microbial pesticides, and organic materials, based on green light illumination techniques.

We hope to develop pest management technology based on LED lighting, broaden its application to many crops, and encourage the use of LEDs in horticulture and plant factories as an eco-friendly light technology.

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